Remarks/Arguments

In response to the Examiner's remarks in the pending Office Action, Applicants have amended claims 48-72, 74-90, 92-119, and added claims 120-127. After entry of this amendment, Claims 48-68, 70-85, 98-100, and 120-127 remain pending. Claims 69, 86-97, and 101-119 are withdrawn.

The application discloses the discovery that the viability of pluripotent or reprogrammed cells created through nuclear transfer is greatly enhanced when the amount of cytoplasm in the donor cytoplast is decreased. This contradicts the previously held understanding in the art that the viability of cells created by nuclear transfer is enhanced when the content of cytoplasm in the oocyte is *increased* rather than *reduced.* The amended claims of the pending application are directed to methods to generate pluripotent or reprogrammed cells by preparing more than one cytoplast fragment from a mammalian oocyte or fertilized zygote and combining a cytoplast *fragment* with a nuclear donor to produce pluripotent or reprogrammed cells.

Amended claim 48 includes the additional limitation that more than one cytoplast fragment be derived from the oocyte or fertilized zygote. This limitation finds support, for example, on page 17, which describes that the amount of intracellular material in each cytoplast "should not be so high that the number of cytoplasts available from one oocyte is limited to one" (paragraph 0061). Support for new claims 120 and 121, which depend from claim 48, is found, for example, on page 17 (paragraph 0061); and support for new claim 122 is found on page 4 (paragraph 0009).

New claims 123 and 124 are directed to a method for reprogramming mammalian cells. Support for these claims is found, for example, on page 5 (paragraph 0010), page 8 (paragraph 0018), page 9 (paragraph 0024), and page 36 (paragraph 0098). Support for new claim 125, which depends from claims 123 and 124, is found, for example, on pages 9-10 (paragraph 0024). The support for new claim 126 which also depends from claim 123, is found, for example, on pages 35-36. Support for new claim 127 is found, for example, on page 8.

In addition, the withdrawn claims have been amended to ensure proper dependency.

The Applicants also present the following Remarks and Arguments in response to the Examiner's comments.

Rejections under 35 U.S.C. § 102(b)

A claim is not anticipated under 35 USC § 102 unless "each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F. 2d 628, 631, 2 USPQ 2d 1051, 1053 (Fed. Cir. 1987).

WO 97/07668

The Examiner has rejected claims 48, 54-67, 70-79, 81, 82, 84, 85, and 98-100 under 35 U.S.C. 102 (b) as anticipated by Campbell *et al.* (PCT Publication No. WO 97/07668, filed August 30, 1996).

The amended claims of the pending application are directed to methods to generate pluripotent or reprogrammed cells by preparing more than one cytoplast fragment from a mammalian oocyte or fertilized zygote and combining a cytoplast *fragment* with a nuclear donor. Campbell *et al.* teaches a method for reconstituting a mammalian embryo by transferring a diploid nucleus into an oocyte that is arrested in metaphase II to produce viable cloned offspring. Campbell *et al.* specifically indicates that techniques should be employed that *increase* the amount of cytoplasm in the oocyte. In particular, pages 9-10 teach several methods that "remove the chromosomes with a minimum of cytoplasm" (page 9, lines 22-23), thereby enucleating the oocyte with minimum disruption to the cytoplasm. Further, Campbell *et al.* provides nuclear transfer methods for the production of reconstituted animal embryos, rather than pluripotent or reprogrammed cells as claimed in the present invention. Thus, Campbell *et al.* does not anticipate the amended claims, and actually teaches away from the claimed invention.

Campbell *et al.* (1993) *Biol. of Repro.* 49:933-942

The Examiner has rejected claims 48, 54, 55, 58, 59, 61-64, 66, 70, 71, 73, 74, 76-82 and 98-100 under 35 U.S.C. 102 (b) as anticipated by Campbell *et al.* (1993) *Biol. of Repro.* 49:933-942. Campbell *et al.* examines nuclear-cytoplasmic interactions during the first cell cycle of nuclear transfer. Campbell *et al.* generates embryos via nuclear transfer techniques, the reference describes that the oocyte is enucleated "by aspirating a *small* amount of cytoplasm from directly beneath the first polar body" (page 935, left column, emphases added). The resulting enucleated, whole oocyte then serves as the recipient for the donor nucleus. Campbell

et al. does not discuss any further modification of the oocyte and does not prepare cytoplast fragments of the oocyte. Thus, Campbell *et al.* does not anticipate the amended claims of the pending application, which require that more than one cytoplast fragment be prepared from the oocyte.

In addition, Campbell *et al.* utilize nuclear transfer to produce reconstituted embryos to examine the influence of the cell cycle stage of both the donor nucleus and the recipient cytoplasm upon the morphology and DNA synthesis potential of the donor nucleus. In contrast, the amended claims of the pending application recite methods to produce pluripotent or reprogrammed cells.

Wolf *et al.* (1998) *J. Biotech.* 65:99-110

The Examiner has rejected claims 48, 54-59, 61-67, 70, 71, 73, 75-82, 84, 85, and 98-100 under 35 U.S.C. 102 (b) as anticipated by Wolf *et al.* (1998) *J. Biotech.* 65:99-110. Wolf *et al.* provides a review of nuclear transfer in mammals and discusses recent developments and future directions. As the Examiner points out, Wolf *et al.* teaches methods of nuclear transfer in mammals, which involves the transfer of genetic material from a donor cell to the cytoplasm of an oocyte or zygote from which the genetic material has been removed, and specifically focuses on reproductive cloning leading to the birth of live offspring. The reference describes a variety of factors that have been shown to influence the viability of offspring. They specifically teach that their studies "using donors with different volumes of cytoplasm provided evidence that a *high karyoplast-cytoplast volume ratio may interfere with development* of bovine nuclear transfer embryos" and cite to Zakhartchenko *et al.* 1997 Mol Reprod Dev 48, 332-338 (page 104, right column). Zakhartchenko *et al.* (copy included in attached Supplemental Information Disclosure Statement) discloses an evaluation of the effects of karyoplast-cytoplast ratio on the development of nuclear transfer embryos. They teach that enucleation of oocytes with a volume similar to that of the respective karyoplast creates better conditions for cell cycle interactions with all types of karyoplasts than enucleation with minimal or large volume of cytoplasm. Thus, Wolf *et al.* and Zakhartchenko *et al.* actually teach away from the claims of the present invention. In addition, Wolf *et al.* describes the effects of varying amounts of cytoplasm on the development of embryos and does not disclose or suggest whether such manipulation would effect the production of pluripotent or reprogrammed mammalian cells. Therefore, Wolf *et al.* does not anticipate the currently amended claims that recite methods to generate pluripotent or reprogrammed cells by

preparing more than one cytoplast fragment from a mammalian oocyte or fertilized zygote and combining a cytoplast *fragment* with a nuclear donor to produce pluripotent or reprogrammed cells.

U.S. Patent No. 5,496,720

The Examiner has rejected claims 48, 54-57, 61-64, 66, 67, 70-74, 76-79, 81, 82, and 98-100 under 35 U.S.C. 102 (b) as anticipated by U.S. Patent No. 5,496,720, filed February 10, 1993 by Susko-Parish *et al.* The '720 patent describes a method of nuclear transfer using parthenogenically activated oocytes as nuclear recipients. The '720 patent teaches that enucleation of the oocyte is conducted by removing the "polar body and a *small* amount of cytoplasm from beneath the polar body". The specification also teaches the parthenogenic activation of the oocytes via increasing the intracellular levels of divalent cations and reducing the phosphorylation of cellular proteins in the oocyte. The whole, enucleated, pathenogenically activated oocytes are then used as nuclear recipients. The specification does not discuss any further modification of the oocyte and does not prepare cytoplast fragments of the oocyte. Further, the '720 patent does not provide any guidance as to whether pluripotent or reprogrammed cells can be produced via the methods described. Thus, the '720 patent does not anticipate the amended claims of the present invention that recite methods to generate pluripotent or reprogrammed cells by (a) preparing more than one cytoplast fragment from a mammalian oocyte or fertilized zygote; (b) obtaining nuclear donor cell or karyoplast taken from a mammal; and (c) combining a cytoplast fragment of step (a) with the nuclear donor cell or karyoplast of step (b) to produce a pluripotent or reprogrammed mammalian cell.

U.S. Patent No. 5,453,366

The Examiner has rejected claims 48, 54-57, 61-64, 66-68, 70-73, 75-79, 81, 82, and 98-100 under 35 U.S.C. 102 (b) as anticipated by U.S. Patent No. 5,453,366, filed March 15, 1993 with priority to July 26, 1990, to Sims and Rosenkrans. The '366 patent describes improved techniques for producing cloned mammalian embryos by positioning the donor embryo nucleus adjacent to the enucleated recipient oocyte, and maintaining the nucleus and oocyte in a maintenance medium for a time sufficient to mature the cytoplasm of the enucleated recipient oocyte prior to fusing the donor nucleus to the recipient oocyte. The '366 patent discloses that

the "cloning procedure includes a *non-disruptive* method of removing the nucleus from a mature recipient oocyte" (col 4, lns 16-18, emphasis added), which is further described as follows:

> "Aspiration applied through the pipette draws a portion of the cytoplasm into the pipette which includes, in the case of the metaphase II bulge, the entire bulge surrounding cytoplasm, or, in the case of the first polar body, the cytoplasm adjacent to the polar body. This process is intended to draw all the metaphase chromosomes into the pipette. As the pipette is withdrawn, with suction maintained, the plasma membrane is stretched and then seals itself leaving a competent plasma membrane on the enucleated oocyte."
>
> (col 7, lines 53-62)

The patent also indicates that the enucleation procedure removes the portion of the cytoplasm containing the nucleus "without, at any point, actually rupturing the plasma membrane" (col 7, lns 45-47). In contrast, the claims of the present invention recite the preparation of cytoplast *fragments* from oocytes or fertilized zygotes.

Furthermore, the '366 patent does not teach the production of pluripotent or reprogrammed cells. The '366 patent indicates (col 9-10, bridging paragraph) that the "embryonic single-cell clones produced as described herein preferably are cultured to the morula or blastula stage," after which they are transplanted into the uteri of suitable animals and grown to term. There is no indication that the embryonic single-cells can produce pluripotent or reprogrammed mammalian cells. Thus, the '366 patent does not anticipate the amended claims of the pending application that recite the production of a pluripotent or reprogrammed mammalian cell.

WO 98/07841

The Examiner has rejected claims 48, 54-60, 62-67, 70, 71, 75-78, 81-85, and 98-100 under 35 U.S.C. 102 (b) as anticipated by Robl *et al.*, WO 98/07841, filed July 28, 1997. Robl *et al.* teaches the production of cross-species embryonic or stem-like cells by transfer of a nucleus into an oocyte from another species. Robl *et al.* describes that the oocyte is enucleated microsurgically using a micropipette to remove the polar body and adjacent cytoplasm. Then a single cell which is heterologous to the enucleated oocyte is transferred into the perivetelline space of the enucleated oocyte to produce a nuclear transfer unit. Thus, the nucleus is transferred into a whole, enucleated oocyte. The specification does not discuss any further modification of the oocyte and does not prepare more than one cytoplast fragment from the oocyte. Hence, Robl

et al. does not anticipate the claims of the pending application that require that more than one cytoplast fragment is prepared from the oocyte.

WO 98/29532

Finally, the Examiner has rejected claims 48-57, 60-64, 66, 67, 70, 71, 72-74, 77, 78, 81, 82, and 98-100 under 35 U.S.C. 102 (b) as anticipated by WO 98/29532, filed December 22, 1997 by Pleura and assigned to Monash University. Pleura specifically addresses the volume of cytoplasm that is donated to a hybrid cell in nuclear transfer procedures. However, contrary to the teachings of the pending application, Pleura indicates the desirability of increasing, rather than decreasing, the levels of cytoplasm contributed by the donor oocyte. Pleura specifically indicates that enhanced cytoplastic content increases the survival of embryos derived from nuclear transfer. Indeed, Pleura teaches that *multiple* cytoplasts be fused to *enhance* the volume of cytoplasm, which is in contrast to the claims of the present invention that recite the use of cytoplast fragments to generate pluripotent or reprogrammed mammalian cells. Thus, Pleura does not anticipate the amended claims of the pending application that recite methods to generate pluripotent or reprogrammed cells by (a) preparing more than one cytoplast fragment from a mammalian oocyte or fertilized zygote; (b) obtaining nuclear donor cell or karyoplast taken from a mammal; and (c) combining a cytoplast fragment of step (a) with the nuclear donor cell or karyoplast of step (b) to produce a pluripotent or reprogrammed mammalian cell.

Claim Rejections under 35 U.S.C. § 112

The Examiner has rejected claims 62-65, 77, and 81 under 35 U.S.C. § 112, second paragraph for failing to particularly point out and distinctly claim the invention. Applicants have amended the claims to address the Examiner's objections, without prejudice or disclaimer.

Claim 62 was held as vague for indicating that the cytoplast donor is "derived from" a non-human species. The Examiner has indicated that the pending claims are vague because it is unclear whether or in what manner the cytoplast donor has been changed from its original source. To address the Examiner's comments, amended claim 62 reads "a cytoplast fragment prepared from a mammalian oocyte or fertilized zygote". Dependant claims 63 and 64 are amended in the same way.

Claim 65 was rejected under 35 U.S.C. § 112 as vague because the claim recites that the nuclear donor is derived from various cells. Claim 65 has been amended to read:

"The method of claim 48, wherein the nuclear donor cell is selected from the group consisting of fibroblasts, granulosa cells, cumulus cells, oviductal epithelium, mammary gland cells, fetal fibroblasts, keratinocytes, hepatocytes, respiratory epithelial cells, neuronal cells, CD34+ stem cells, granulocytes, ~~or~~ and mononuclear peripheral blood cells."

The Examiner has rejected claim 77 as unclear because of the term "cell/karyoplast." The claim has been amended to recite an "embryonic, fetal, or adult cell, or embryonic, fetal, or adult karyoplast."

The Examiner has also rejected claim 81 as unclear as it recites a "differentiated stem cell." Amended claim 81 more clearly reads that the "nuclear donor is from a stem cell, or differentiated or undifferentiated somatic cell."

In view of the amendments and remarks herein, withdrawal of the outstanding rejections is requested. Applicants believe that the Examiner's objections to the specification and claims have been addressed. In addition, Applicants have taken into consideration the Examiner's rejections and believe that the amendments and arguments place the application into condition for allowance. Please charge any additional fees required or credit any overpayments to Deposit Account No. 11-0980.

Respectfully submitted,
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